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 NEWS  1          Web Page for STN Seminar Schedule - N. America
 NEWS  2  AUG 06  CAS REGISTRY enhanced with new experimental property tags
 NEWS  3  AUG 06  FSTA enhanced with new thesaurus edition
 NEWS  4  AUG 13  CA/CAplus enhanced with additional kind codes for granted
                  patents
 NEWS  5  AUG 20  CA/CAplus enhanced with CAS indexing in pre-1907 records
 NEWS  6  AUG 27  Full-text patent databases enhanced with predefined
                  patent family display formats from INPADOCDB
 NEWS  7  AUG 27  USPATOLD now available on STN
 NEWS  8  AUG 28  CAS REGISTRY enhanced with additional experimental
                  spectral property data
 NEWS  9  SEP 07  STN AnaVist, Version 2.0, now available with Derwent
                  World Patents Index
 NEWS 10  SEP 13  FORIS renamed to SOFIS
 NEWS 11  SEP 13  INPADOCDB enhanced with monthly SDI frequency
 NEWS 12  SEP 17  CA/CAplus enhanced with printed CA page images from
                  1967-1998
 NEWS 13  SEP 17  CAplus coverage extended to include traditional medicine
                  patents
 NEWS 14  SEP 24  EMBASE, EMBAL, and LEMBASE reloaded with enhancements
 NEWS 15  OCT 02  CA/CAplus enhanced with pre-1907 records from Chemisches
                  Zentralblatt
 NEWS 16  OCT 19  BEILSTEIN updated with new compounds
 NEWS 17  NOV 15  Derwent Indian patent publication number format enhanced
 NEWS 18  NOV 19  WPIX enhanced with XML display format
 NEWS 19  NOV 30  ICSD reloaded with enhancements
 NEWS 20  DEC 04  LINPADOCDB now available on STN
 NEWS 21  DEC 14  BEILSTEIN pricing structure to change
 NEWS 22  DEC 17  USPATOLD added to additional database clusters
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 NEWS 23  DEC 17  IMSDRUGCONF removed from database clusters and STN
 NEWS 24  DEC 17  DGENE now includes more than 10 million sequences
 NEWS 25  DEC 17  TOXCENTER enhanced with 2008 MeSH vocabulary in
                  MEDLINE segment
 NEWS 26  DEC 17  MEDLINE and LMEDLINE updated with 2008 MeSH vocabulary
 NEWS 27  DEC 17  CA/CAplus enhanced with new custom IPC display formats
 NEWS 28  DEC 17  STN Viewer enhanced with full-text patent content
                  from USPATOLD

 NEWS EXPRESS  19 SEPTEMBER 2007:  CURRENT WINDOWS VERSION IS V8.2,
               CURRENT MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
               AND CURRENT DISCOVER FILE IS DATED 19 SEPTEMBER 2007.

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=> File  .Gerry2MBCE
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FULL ESTIMATED COST                                       0.21       0.21

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=> S (Immobilized Metal affinity chromatography)(S)(Copper OR Cu)AND pd<=20050515
   2 FILES SEARCHED...
L1         116 (IMMOBILIZED METAL AFFINITY CHROMATOGRAPHY)(S)(COPPER OR CU)
               AND PD<=20050515

=> S L1 AND (Purification OR isolation)
L2          55 L1 AND (PURIFICATION OR ISOLATION)

=> Dup rem L2
PROCESSING COMPLETED FOR L2
L3           45 DUP REM L2 (10 DUPLICATES REMOVED)
                ANSWERS '1-7' FROM FILE MEDLINE
                ANSWERS '8-12' FROM FILE BIOSIS
                ANSWERS '13-40' FROM FILE CAPLUS
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ANSWERS '41-45' FROM FILE EMBASE

=> D ti L3 1-45

L3 ANSWER 1 OF 45 MEDLINE on STN DUPLICATE 1
TI Separation of native prion protein (PrP) glycoforms by copper -binding using immobilized metal affinity chromatography (IMAC).

L3 ANSWER 2 OF 45 MEDLINE on STN DUPLICATE 8
TI Purification of a recombinant human respiratory syncytial virus chimeric glycoprotein using reversed-phase chromatography and protein refolding in guanidine hydrochloride.

L3 ANSWER 3 OF 45 MEDLINE on STN DUPLICATE 9
TI Large-scale immunoaffinity purification of recombinant soluble human antigen CD4 from Escherichia coli cells.

L3 ANSWER 4 OF 45 MEDLINE on STN
TI Evaluating immobilized metal affinity chromatography for the selection of histidine-containing peptides in comparative proteomics.

L3 ANSWER 5 OF 45 MEDLINE on STN
TI Protein proteolysis and the multi-dimensional electrochromatographic separation of histidine-containing peptide fragments on a chip.

L3 ANSWER 6 OF 45 MEDLINE on STN
TI Structural damage to lactate dehydrogenase during copper iminodiacetic acid metal affinity chromatography.

L3 ANSWER 7 OF 45 MEDLINE on STN
TI Preparative separation of small molecular weight peptides from casein hydrolysate using gel filtration and immobilized metal ion affinity chromatography.

L3 ANSWER 8 OF 45 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 2
TI Separation of native prion protein (PrP) glycoforms by copper -binding using immobilized metal affinity chromatography (IMAC).

L3 ANSWER 9 OF 45 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 6
TI Evaluation of immobilized metal affinity chromatography (IMAC) for isolation and recovery of strong copper-complexing ligands from marine waters.

L3 ANSWER 10 OF 45 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 7
TI Copper ligands isolated from estuarine water by immobilized metal affinity chromatography: Temporal variability and partial characterization.

L3 ANSWER 11 OF 45 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
TI Isolation and separation of alpha-amylase inhibitors I-1 and I-2 from seeds of ragi (Indian finger millet, Eleusine coracana) by metal chelate affinity precipitation.

L3 ANSWER 12 OF 45 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
TI Purification of P0 myelin glycoprotein for crystallization.

L3 ANSWER 13 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 3
TI Purification of copper-zinc superoxide dismutase from Semen Lethospermi (SL) by copper-chelate affinity chromatography

L3 ANSWER 14 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 4
TI Purification of P0 myelin glycoprotein by a Cu2+ -immobilized metal affinity chromatography

L3 ANSWER 15 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 5
TI One-step purification of urease from Canavalia ensiformis by immobilized metal affinity chromatography

L3 ANSWER 16 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN
TI Analysis of complex mixtures of proteins by sequencing of subsets of peptide fragments derived from the mixture

L3 ANSWER 17 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN
TI Molecular dynamics simulations of metal ion binding to the His-tag motif

L3 ANSWER 18 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN
TI Protein C production: metal ion/protein interfacial interaction in immobilized metal affinity chromatography

L3 ANSWER 19 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN
TI Use of immobilized metal affinity chromatography as a capture mode for purification of a therapeutic protein product

L3 ANSWER 20 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN
TI Process for the purification of TNF-binding proteins using IMAC

L3 ANSWER 21 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN
TI Purification of goat immunoglobulin G by immobilized metal-ion affinity using cross-linked alginate beads

L3 ANSWER 22 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN
TI Nucleic acid separations utilizing immobilized metal affinity chromatography

L3 ANSWER 23 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN
TI Analysis of equilibrium adsorption isotherms for human protein C purification by immobilized metal affinity chromatography

L3 ANSWER 24 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN
TI Purification of recombinant BtpA and Ycf3, proteins involved in membrane protein biogenesis in Synechocystis PCC 6803

L3 ANSWER 25 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN
TI Thermosensitive copolymer of N-vinylcaprolactam and 1-vinylimidazole: molecular characterization and separation by immobilized metal affinity chromatography

L3 ANSWER 26 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN
TI Characterization of metal affinity of green fluorescent protein and its purification through salt promoted, immobilized metal affinity chromatography

L3 ANSWER 27 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN
TI Immobilized metal affinity chromatography of β-galactosidase from unclarified Escherichia coli homogenates using expanded bed adsorption

L3 ANSWER 28 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN

TI Comparison of different transition metal ions for immobilized metal affinity chromatography of selenoprotein P from human plasma

L3 ANSWER 29 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN
TI Purification of papain by immobilized metal affinity chromatography (IMAC) on chelating carboxymethyl cellulose

L3 ANSWER 30 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN
TI Purification of coniferyl alcohol oxidase from lignifying xylem of Sitka spruce using immobilized metal affinity chromatography

L3 ANSWER 31 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN
TI Purification of hemocyanin from white shrimp (Penaeus vannamei Boone) by immobilized metal affinity chromatography

L3 ANSWER 32 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN
TI Selection of optimum affinity tags from a phage-displayed peptide library. Application to immobilized copper(II) affinity chromatography

L3 ANSWER 33 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN
TI Purification of human erythrocyte acetylcholinesterase by immobilized metal affinity chromatography

L3 ANSWER 34 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN
TI Salicylaldehyde-metal-amino acid ternary complex: a new tool for immobilized metal affinity chromatography

L3 ANSWER 35 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN
TI Fractionation of perforin and granzymes by immobilized metal affinity chromatography (IMAC)

L3 ANSWER 36 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN
TI Method for purification of a mixture of hydroxamate derivatized protein and native protein

L3 ANSWER 37 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN
TI Immobilized metal affinity membrane adsorbers as stationary phases for metal interaction protein separation

L3 ANSWER 38 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN
TI Resolution of three forms of superoxide dismutase by immobilized metal affinity chromatography

L3 ANSWER 39 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN
TI Rapid analytical and preparative scale immobilized metal affinity chromatography to purify α-lactalbumin from milk whey

L3 ANSWER 40 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN
TI Separation of ovotransferrin from egg white by immobilized metal affinity chromatography

L3 ANSWER 41 OF 45 EMBASE COPYRIGHT (c) 2007 Elsevier B.V. All rights reserved on STN
TI Macroporous chitosan layer coated on non-porous silica gel as a support for metal chelate affinity chromatographic adsorbent.

L3 ANSWER 42 OF 45 EMBASE COPYRIGHT (c) 2007 Elsevier B.V. All rights reserved on STN
TI Efficient two-step chromatographic purification of penicillin acylase from clarified Escherichia coli ultrasonic homogenate.

L3 ANSWER 43 OF 45 EMBASE COPYRIGHT (c) 2007 Elsevier B.V. All rights

reserved on STN
TI Purification and characterization of lymphocyte chymase I, a granzyme implicated in perforin-mediated lysis.

L3 ANSWER 44 OF 45 EMBASE COPYRIGHT (c) 2007 Elsevier B.V. All rights reserved on STN
TI Chromatographic behaviour of amidases from strains of Pseudomonas aeruginosa on immobilized metal chelates.

L3 ANSWER 45 OF 45 EMBASE COPYRIGHT (c) 2007 Elsevier B.V. All rights reserved on STN
TI Perforin-enhancing protein, a low molecular weight protein of cytotoxic lymphocyte granules, enhances perforin lysis.

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FULL ESTIMATED COST	32.54	32.75

=> D hist

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FILE 'MEDLINE, BIOSIS, CAPLUS, EMBASE' ENTERED AT 15:48:48 ON 18 DEC 2007
L1 116 S (IMMOBILIZED METAL AFFINITY CHROMATOGRAPHY)(S)(COPPER OR CU)A
L2 55 S L1 AND (PURIFICATION OR ISOLATION)
L3 45 DUP REM L2 (10 DUPLICATES REMOVED)

=> D ibib abs L3 1-5, 7, 11, 13, 14, 16-20, 23, 24, 26-29, 32-40, 42-45

L3 ANSWER 1 OF 45 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2005240393 IN-PROCESS
DOCUMENT NUMBER: PubMed ID: 15658935
TITLE: Separation of native prion protein (PrP) glycoforms by copper-binding using immobilized metal affinity chromatography (IMAC).

AUTHOR: Muller Henrik; Strom Alexander; Hunsmann Gerhard; Stuke Andreas W
CORPORATE SOURCE: Institut fur Physikalische Biologie, Heinrich-Heine-Universitat Dusseldorf, Universitatsstr. 1, D-40225 Dusseldorf, Germany.
SOURCE: The Biochemical journal, (2005 May 15) Vol. 388, No. Pt 1, pp. 371-8.
Journal code: 2984726R. E-ISSN: 1470-8728.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE) (RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: NONMEDLINE; IN-PROCESS; NONINDEXED; Priority Journals
ENTRY DATE: Entered STN: 10 May 2005
Last Updated on STN: 14 Dec 2005

AB The conformational conversion of the normal cellular prion protein (PrPC) into the pathology-associated PrPSc isoform is a key event in TSEs (transmissible spongiform encephalopathies). The host PrPC molecule contains two N-linked glycosylation sites and binds copper under physiological conditions. In contrast with PrPC, PrPSc is insoluble in non-ionic detergents and does not bind to Cu2+ ions. Hence, we utilized copper binding to separate and characterize both PrP isoforms. Infected and uninfected murine brain and bovine stem brain specimens were treated with the mild non-ionic detergent n-octyl-beta-D-glucopyranoside (octylglucoside) to maintain the native PrP conformations during isolation. The solubilized homogenates were loaded on to Cu2+-saturated IMAC (immobilized metal affinity chromatography) columns and eluted using the chelating agent EDTA. Fractions were separated by SDS/PAGE and analysed by immunoblotting using anti-PrP monoclonal antibodies for glycosylation profiling. Whereas native PrPC and denatured PrPSc were retained by a Cu2+-loaded resin, native PrPSc and PrPres [PK (proteinase K)-resistant PrP] passed through the column. We demonstrate here that the IMAC technique is appropriate to isolate and partially purify PrPC from healthy brains in its native-like and biologically relevant glycosylated copper-binding forms. The IMAC technique is also well suited for the separation of native PrPC from aggregated PrPSc in infected brains. Our results indicate that in contrast with PrPSc in uninfected as well as infected brains, PrPC is predominantly present in the glycosylated forms.

L3 ANSWER 2 OF 45 MEDLINE on STN DUPLICATE 8
ACCESSION NUMBER: 95037721 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7950387
TITLE: Purification of a recombinant human respiratory syncytial virus chimeric glycoprotein using reversed-phase chromatography and protein refolding in guanidine hydrochloride.
AUTHOR: Wells P A; Garlick R L; Lyle S B; Tuls J L; Poorman R A; Brideau R J; Wathen M W
CORPORATE SOURCE: Upjohn Company, Kalamazoo, Michigan 49001.
SOURCE: Protein expression and purification, (1994 Aug) Vol. 5, No. 4, pp. 391-401.
Journal code: 9101496. ISSN: 1046-5928.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199412
ENTRY DATE: Entered STN: 10 Jan 1995
Last Updated on STN: 6 Feb 1998
Entered Medline: 29 Dec 1994

AB FG glycoprotein is a recombinant chimeric protein consisting of the

extracellular portions of human respiratory syncytial virus (RSV) F and G glycoproteins. In theory, highly purified FG glycoprotein may be effective as a RSV vaccine. Recombinant FG glycoprotein was expressed using the baculovirus/insect cell system. FG glycoprotein was isolated from cell culture supernatants using S Sepharose ion-exchange chromatography, Cu(2+)-immobilized metal affinity chromatography, preparative reversed-phase high-performance liquid chromatography, denaturation with 6 M guanidine hydrochloride, and protein refolding in Tween 80 detergent. The purified FG glycoprotein was concentrated on a S Sepharose column and exchanged into an appropriate buffer for vaccine formulation. Five batches of FG glycoprotein with protein purity of 92-99% were produced using this purification process. FG glycoprotein produced using reversed-phase chromatography and protein refolding was compared with nondenatured FG glycoprotein using a panel of 14 monoclonal antibodies directed against conformational and linear epitopes on RSV F and G glycoproteins. The results of these studies indicated that refolded FG glycoprotein had the same three-dimensional structure as nondenatured FG glycoprotein.

L3 ANSWER 3 OF 45 MEDLINE on STN DUPLICATE 9
ACCESSION NUMBER: 94128252 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8297511
TITLE: Large-scale immunoaffinity purification of recombinant soluble human antigen CD4 from Escherichia coli cells.
AUTHOR: Wells P A; Beiderman B; Garlick R L; Lyle S B; Martin J P Jr; Herberg J T; Meyer H F; Henderson S L; Eckenrode F M
CORPORATE SOURCE: Cancer and Infectious Diseases Research Services, Upjohn Company, Kalamazoo, MI 49001.
SOURCE: Biotechnology and applied biochemistry, (1993 Dec) Vol. 18 (Pt 3), pp. 341-57.
Journal code: 8609465. ISSN: 0885-4513.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199403
ENTRY DATE: Entered STN: 18 Mar 1994
Last Updated on STN: 3 Mar 2000
Entered Medline: 10 Mar 1994

AB A large-scale immunoaffinity (IA) purification process was developed for the isolation of recombinant soluble antigen CD4 (sCD4) from Escherichia coli fermentations. The monoclonal antibody used for IA purification of sCD4 recognized a conformation-dependent epitope on the surface of domain 1 of CD4. IA chromatography was used to purify both sCD4-183, consisting of the N-terminal 183 amino acids of human CD4, and sCD4-PE40, a fusion protein consisting of the N-terminal 178 amino acids of CD4 and amino acids 1-3 and 253-613 of Pseudomonas exotoxin A (PE40). sCD4-183 was purified from E. coli cell pellets using cell disruption, protein solubilization, oxidation, Q-Sepharose anion-exchange and IA chromatography steps. sCD4-PE40 was purified from cell pellets using cell disruption, protein solubilization, oxidation, Cu(2+)-immobilized metal-affinity chromatography, anion-exchange and IA chromatography steps. The IA-purified sCD4 analogues demonstrated the correct apparent molecular masses on SDS/PAGE. The immobilized monoclonal antibody appeared to select for correctly folded CD4 protein, since sCD4-183 and sCD4-PE40 purified by the IA method bound human-immunodeficiency-virus glycoprotein gp120 (HIV gp120) in vitro. sCD4-PE40 purified by IA chromatography also inhibited protein synthesis in CV-1 cells expressing HIV gp120/160 at the cell surface. Relatively high recoveries of sCD4-183 and sCD4-PE40 were

observed in the IA step of the purification process (71 and 79% recovery respectively). The results demonstrate that immobilized monoclonal antibodies directed against conformational epitopes may be used for rapid purification of gram amounts of correctly folded protein from mixtures of oxidized E. coli proteins.

L3 ANSWER 4 OF 45 MEDLINE on STN
ACCESSION NUMBER: 2003286725 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12814271
TITLE: Evaluating immobilized metal affinity chromatography for the selection of histidine-containing peptides in comparative proteomics.
AUTHOR: Ren Diya; Penner Natalia A; Slentz Benjamin E; Mirzaei Hamid; Regnier Fred
CORPORATE SOURCE: Department of Chemistry, Purdue University, West Lafayette, Indiana 47907, USA.
CONTRACT NUMBER: 5R01 GM 5996-04 (NIGMS)
SOURCE: Journal of proteome research, (2003 May-Jun) Vol. 2, No. 3, pp. 321-9.
Journal code: 101128775. ISSN: 1535-3893.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE) (RESEARCH SUPPORT, U.S. GOV'T, P.H.S.)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200402
ENTRY DATE: Entered STN: 20 Jun 2003
Last Updated on STN: 6 Feb 2004
Entered Medline: 5 Feb 2004
AB Agarose based immobilized metal affinity chromatography (IMAC) columns loaded with copper (II) were evaluated for the selection of histidine-containing peptides in comparative proteomics. Recovery, binding specificity, and reproducibility were investigated with model proteins. Cu(II)-IMAC was found to be highly selective for histidine containing peptides; moreover, a low degree of nonspecific selection was observed. Acylation of the amino-terminus of peptides with either succinic anhydride, N-acetoxysuccinamide, or [3-(2,5)-dioxopyrrolidin-1-yloxycarbonyl)-propyl]-trimethylammonium (quaternary amine) reduced the number of histidine-containing peptides bound by the Cu(II)-IMAC columns. This provides an additional possibility for sample simplification in proteomic applications. The number of acylated peptides selected decreased in the order of quaternary amine > N-acetoxysuccinamide > succinic anhydride derivatization. Although the selection of N-terminally derivatized peptides is biased toward peptides that contain more than one histidine, it is not yet possible to predict selectivity.

L3 ANSWER 5 OF 45 MEDLINE on STN
ACCESSION NUMBER: 2003054409 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12564680
TITLE: Protein proteolysis and the multi-dimensional electrochromatographic separation of histidine-containing peptide fragments on a chip.
AUTHOR: Slentz Benjamin E; Penner Natalia A; Regnier Fred E
CORPORATE SOURCE: Department of Chemistry, Purdue University, 1393 Brown Building, West Lafayette, IN 47907-1393, USA.
CONTRACT NUMBER: 57667
59996
SOURCE: Journal of chromatography. A, (2003 Jan 10) Vol. 984, No. 1, pp. 97-107.
Journal code: 9318488. ISSN: 0021-9673.
PUB. COUNTRY: Netherlands

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, U.S. GOV'T, P.H.S.)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200307
ENTRY DATE: Entered STN: 5 Feb 2003
Last Updated on STN: 17 Jul 2003
Entered Medline: 16 Jul 2003

AB This paper reports a system for three-dimensional electrochromatography in a chip format. The steps involved included trypsin digestion, copper(II)-immobilized metal affinity chromatography [Cu(II)-IMAC] selection of histidine-containing peptides, and reversed-phase capillary electrochromatography of the selected peptides. Trypsin digestion and affinity chromatography were achieved in particle-based columns with a microfabricated frit whereas reversed-phase separations were executed on a column of collocated monolithic support structures. Column frits were designed to maintain constant cross sectional area and path length in all channels and to retain particles down to a size of 3 microm. Cu(II)-IMAC selection of histidine-containing peptides from standard peptide mixtures and protein digests followed by reversed-phase chromatography of the selected peptides was demonstrated in the electrochromatography mode. The possibility to run a comprehensive proteomic analysis by combining trypsin digestion, affinity selection, and a reversed-phase separation on chips was shown using fluorescein isothiocyanate-labeled bovine serum albumin as an example.

L3 ANSWER 7 OF 45 MEDLINE on STN
ACCESSION NUMBER: 90311013 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2367347
TITLE: Preparative separation of small molecular weight peptides from casein hydrolysate using gel filtration and immobilized metal ion affinity chromatography.
AUTHOR: Gauthier J; Amiot J; Vijayalakshmi M A
CORPORATE SOURCE: Departement de Sciences et Technologie des Aliments, Universite Laval, Ste-Foy, Quebec.
SOURCE: Preparative biochemistry, (1990) Vol. 20, No. 1, pp. 23-50.
Journal code: 1276634. ISSN: 0032-7484.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199008
ENTRY DATE: Entered STN: 21 Sep 1990
Last Updated on STN: 29 Jan 1996
Entered Medline: 13 Aug 1990

AB A two step method consisting of a gel filtration step, followed by a Immobilized Metal Affinity Chromatography (IMAC) step using a IDA-Cu coupled Sephadex G-25 column, on a preparative scale is described for the group separation of peptides from a casein hydrolysate. The 48 groups of peptides thus separated are further characterised by RP-HPLC and amino acid analysis. Some peptides after the analytical RP-HPLC step are further characterised by sequencing. An insight into the mechanism of retention on IMAC of the peptides is attempted. In such complex mixtures as casein hydrolysate, the peptide-peptide interaction can mask the potential sites of interactions in a single peptide. The results obtained using volatile buffers as eluents show the possibility of using IMAC step as an alternative to obtain gram quantities of group of peptides free of salts from complex protein hydrolysates.

L3 ANSWER 11 OF 45 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
ACCESSION NUMBER: 1999:135058 BIOSIS
DOCUMENT NUMBER: PREV199900135058
TITLE: Isolation and separation of alpha-amylase inhibitors I-1 and I-2 from seeds of ragi (Indian finger millet, Eleusine coracana) by metal chelate affinity precipitation.
AUTHOR(S): Kumar, A.; Galaev, I. Yu.; Mattiasson, B. [Reprint author]
CORPORATE SOURCE: Dep. Biotechnol., Cent. Chem. Chem. Eng., Lund Univ., P.O. Box 124, Lund S-22100, Sweden
SOURCE: Bioseparation, (1998) Vol. 7, No. 3, pp. 129-136. print.
ISSN: 0923-179X.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 31 Mar 1999
Last Updated on STN: 31 Mar 1999
AB The concept of immobilized metal affinity chromatography (IMAC) was integrated with affinity precipitation for the single step isolation of alpha-amylase inhibitors I-1 and 1-2 from the seeds of ragi (Indian finger millet, Eleusine coracana). alpha-Amylase inhibitor I-1 was purified 13-fold with a yield of 84%, using Cu(II) loaded thermosensitive metal chelate copolymer of N-isopropylacrylamide (NIPAM) and 1-vinyl imidazole (VI). The protein also showed trypsin inhibitory activity. The binding of the protein to the copolymer was strongly pH dependent. alpha-Amylase inhibitor 1-2 was recovered in the supernatant as unprecipitated protein with significant purification and constituted 27% of the total inhibitor power. The yield with respect to inhibitor 1-2 was around 85%. Sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) showed significant purification of inhibitor I-1 and indicated evident separation of the two proteins on metal chelate affinity precipitation.

L3 ANSWER 13 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 3
ACCESSION NUMBER: 2005:36988 CAPLUS
DOCUMENT NUMBER: 142:275851
TITLE: Purification of copper-zinc superoxide dismutase from Semen Lethospermi (SL) by copper-chelate affinity chromatography
AUTHOR(S): Haddad, Namir I. A.; Yuan, Qin-Sheng
CORPORATE SOURCE: State Key Laboratory of Bioreactor Engineering and School of Bio-engineering, East China University of Science and Technology, Shanghai, 200237, Peop. Rep. China
SOURCE: Zhongguo Tianran Yaowu (2004), 2(6), 379-384
CODEN: ZTYHA7; ISSN: 1672-3651
PUBLISHER: Zhongguo Tianran Yaowu Bianjibu
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A new method for separation of Cu/Zn superoxide dismutase (CuZn-SOD) from the Chinese traditional medicine Semen Lethospermi, including immobilized metal affinity chromatog
. (INIAC), is reported. A multi-elution technique was used in Cu-chelate affinity chromatog.; increasing and decreasing the pH gradient, followed by gradient elution with histidine resulted in a high resolution of the enzyme. CuZn-SOD appeared to be homogeneous as judged by PAGE.
REFERENCE COUNT: 33 THERE ARE 33 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 14 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 4
ACCESSION NUMBER: 1999:383920 CAPLUS

DOCUMENT NUMBER: 131:167245
TITLE: Purification of P0 myelin glycoprotein by a Cu2+ -immobilized metal affinity chromatography
AUTHOR(S): Sedzik, Jan; Kotake, Yoshiko; Uyemura, Keiichi
CORPORATE SOURCE: Department of Physiology, Keio University School of Medicine, Tokyo, 160-8582, Japan
SOURCE: Neurochemical Research (1999), 24(6), 723-732
CODEN: NEREDZ; ISSN: 0364-3190
PUBLISHER: Kluwer Academic/Plenum Publishers
DOCUMENT TYPE: Journal
LANGUAGE: English

AB P0 is an abundant myelin glycoprotein of peripheral nerves of vertebrates. Various point mutations of this protein are responsible for hereditary neuropathies. In this paper we described purification of P0 glycoprotein using SDS and a metal chelate affinity chromatog. Purified myelin fraction from bovine spinal roots in 0.5% SDS, 0.5 M NaCl, 50 mM Tris-HCl, pH 7.4 is filtered and applied directly to the Cu2+-immobilized affinity chromatog. column, equilibrated with the same buffer. After eluting a void volume (or pass through) fraction, P0 protein was eluted by the same buffer but without salt. To remove contamination from the eluent, further purification is continued on a Concanavalin-A coupled agarose column. We purify within two days, 30 mg of P0 protein of apparent mol. weight 27 kDa. The method can be used to purify recombinant or mutated P0 protein found in severe pathologies.

REFERENCE COUNT: 65 THERE ARE 65 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 16 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2005:120658 CAPLUS
DOCUMENT NUMBER: 142:214859
TITLE: Analysis of complex mixtures of proteins by sequencing of subsets of peptide fragments derived from the mixture
INVENTOR(S): Regnier, Fred E.; Ademec, Jiri; Zhang, Xiang
PATENT ASSIGNEE(S): Beyond Genomics, Inc., USA
SOURCE: PCT Int. Appl., 140 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2005011576	A2	20050210	WO 2004-US23908	20040723 <--
WO 2005011576	A3	20050506		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 2003-490149P P 20030725

AB A method of analyzing a protein mixture by anal. of peptides derived from the mixture, e.g. tryptic fragments, is described. A protein mixture is converted to peptides and fractionated. Peptides with specific features

may be isolated in different ways: cysteine- containing peptides may be captured with avidin, histidine-containing peptides may be captured by immobilized metal affinity chromatog
. with Cu(II) and acidic peptides may be captured with a strong anion exchanger. Fractionating peptide mixts. by combinations of such methods can generate pools of relatively low complexity suitable for high throughput sequencing. These fractions, none of which are necessarily fully representative of the original protein mixture, are analyzed, e.g. by mass spectrometry, and amino acid sequences of individual peptides within the mixture are determined Sequence data from the peptides are used to determine the
likelihood of the presence of two or more proteins in the mixture In one embodiment, the peptide selection approach selects peptides such that two or more sets of peptides are each individually non-representative of the protein mixture In another embodiment, peptides are selected such that two or more sets of peptides are each individually representative of less than about 70% but greater than about 30% of the proteins in the protein mixture

L3 ANSWER 17 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2006:174628 CAPLUS
DOCUMENT NUMBER: 145:98984
TITLE: Molecular dynamics simulations of metal ion binding to the His-tag motif
AUTHOR(S): Chen, Chin-Wen; Liu, Hsuan-Liang; Lin, Jin-Chung; Ho, Yih
CORPORATE SOURCE: Graduate Institute of Biotechnology, National Taipei University of Technology, Taipei, 10608, Taiwan
SOURCE: Journal of the Chinese Chemical Society (Taipei, Taiwan) (2005), 52(6), 1281-1290
CODEN: JCCTAC; ISSN: 0009-4536
PUBLISHER: Chinese Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English
AB In our previous study, we have observed that the chelation of various metal ions to the His-tag motifs mostly involves the i and i+2 His residues for Ni2+, Cu2+, Zn2+ and Co2+. In the present study, various 200 ps mol. dynamics simulations were further conducted to investigate the chelating pathway of various metal ions to the His-tag motif with 6 His residues (His-tag6) and the binding affinities of these metal binding pockets towards these metal ions. The results indicate that His-tag6 with the chelated metal ion located in positions His(2,4) or His(3,5) exhibits the strongest affinity for Ni2+ and Cu2+. K+ was found to be preferred to chelate in His(1,3) and His(3,5) coordinations. However, Fe3+ was found to have higher affinity towards His(1,3) and His(2,4) binding pockets. Our results also suggest that Ni2+ exhibits the highest binding affinity towards His-tag6 over the other metal ions. Most of the structural variations of the His-tag6 motif were from the Histidyl side chains during metal ion binding. In addition, there is an inverse linear correlation between the final chelated distance and the charge/volume ratio of metal ion. There is a neg. correlation between the metal binding affinity and the averaged potential energy generated from the MD simulations.
REFERENCE COUNT: 41 THERE ARE 41 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 18 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2007:859858 CAPLUS
TITLE: Protein C production: metal ion/protein interfacial interaction in immobilized metal affinity chromatography
AUTHOR(S): Lee, James J.; Thiessen, Eileen; Bruley, Duane F.
CORPORATE SOURCE: USA
SOURCE: Advances in Experimental Medicine and Biology (

2005), 566(Oxygen Transport to Tissue XXVI), 381-387
CODEN: AEMBAP; ISSN: 0065-2598
PUBLISHER: Springer
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Protein C (PC) is an essential blood factor in the human blood coagulation cascade. PC can help achieve blood hemostasis in many deadly disease conditions such as sepsis, cancer, HIV, etc.; reduced oxygen transport due to blood agglutination within the body can cause tissue death and organ failure as a result of low oxygen transport. Our goal is to produce large quantities of low cost zymogen PC for the treatment and prevention of blood clotting resulting from many disease states, as well as provide an effective therapy for PC deficiency. Current studies show that Immobilized Metal Affinity Chromatog. (IMAC) has high specificity and can be used for difficult sepns. among homologous proteins at relatively low cost compared to current methods, such as Immunoaffinity Chromatog. Thus, we are investigating the optimization of IMAC for the separation and purification of PC from Cohn fraction IV-I. Mol. interactions within the chromatog. column involve many parameters that include: the use and type of chromatog. gel and buffer solution, the pH, temperature, metal ion, chelator, and the sequence and structure of the protein itself. These parameters all influence the protein's interaction with the column. Exptl. equilibrium isotherms show that PC has primary and secondary binding characteristics, demonstrating that the interaction is not just a simple process of one protein binding to one metal ion. Understanding the thermodn. of interfacial interaction between proteins and surface-bound Cu2+ is essential to optimizing IMAC for PC purification, as well as for separation of other proteins in general. Hence we are undertaking theor. and exptl. studies of IDA-Cu/PC adsorption. The differences in structures of PC and other critical homologous blood factors are examined using the protein visualization program Cn3D. A better understanding of the interfacial phenomena will help determine the most effective conditions to achieve our goal.
REFERENCE COUNT: 19 THERE ARE 19 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 19 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2005:186356 CAPLUS
TITLE: Use of immobilized metal affinity chromatography as a capture mode for purification of a therapeutic protein product
AUTHOR(S): Rathore, Anurag; Hong, Tony; Dransart, Bryan; Tressel, Tim
CORPORATE SOURCE: Process Development, Amgen, Thousand Oaks, CA, 91320, USA
SOURCE: Abstracts of Papers, 229th ACS National Meeting, San Diego, CA, United States, March 13-17, 2005 (2005), BIOT-115. American Chemical Society: Washington, D. C.
CODEN: 69GQMP
DOCUMENT TYPE: Conference; Meeting Abstract
LANGUAGE: English
AB Immobilized metal affinity chromatog . (IMAC) is a mode of chromatog. that is based on complexation that occurs between certain amino acids (e.g. histidine, cysteine, and tryptophan) and the transition metal ions (e.g. copper, zinc, nickel). While not commonly used as some other modes of chromatog., for certain applications IMAC offers a unique combination of high recovery, selectivity and robustness. This talk will focus on an application that involves use of IMAC for post-harvest capture of a protein product. Effects of different process parameters on step performance were evaluated

as part of the optimization studies. This was followed by characterization studies to examine the robustness of the IMAC step and scale-up for clin. manufacturing The results presented in this talk indicate that the IMAC step, if optimally designed, is capable of offering a high yielding, highly specific and scale-able separation

L3 ANSWER 20 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2004:453247 CAPLUS
DOCUMENT NUMBER: 140:420337
TITLE: Process for the purification of TNF-binding proteins using IMAC
INVENTOR(S): Rossi, Mara
PATENT ASSIGNEE(S): Ares Trading S.A., Switz.
SOURCE: PCT Int. Appl., 39 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2004046184	A1	20040603	WO 2003-EP50824	20031113 <--
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
CA 2505385	A1	20040603	CA 2003-2505385	20031113 <--
AU 2003298287	A1	20040615	AU 2003-298287	20031113 <--
EP 1560851	A1	20050810	EP 2003-796015	20031113
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK				
BR 2003015807	A	20050920	BR 2003-15807	20031113
CN 1738835	A	20060222	CN 2003-80108609	20031113
JP 2006517187	T	20060720	JP 2004-552721	20031113
ZA 2005003702	A	20060830	ZA 2005-3702	20050509
BG 109152	A	20060428	BG 2005-109152	20050510
MX 2005PA05246	A	20050725	MX 2005-PA5246	20050516
NO 2005002916	A	20050615	NO 2005-2916	20050615
US 2006128616	A1	20060615	US 2005-534535	20050908
PRIORITY APPLN. INFO.:			EP 2002-25755 A	20021115
			WO 2003-EP50824 W	20031113

AB A new purification process for Tumor Necrosis Factor-binding proteins is described. In particular this process is characterized by the use as capture step of an Immobilized Metal Affinity Chromatog. (IMAC) using copper as metal. This brings advantages in terms of process yields, purity of the final product and applicability to industrial scale.

L3 ANSWER 23 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2004:916227 CAPLUS
DOCUMENT NUMBER: 142:478139
TITLE: Analysis of equilibrium adsorption isotherms for human protein C purification by immobilized metal affinity chromatography
AUTHOR(S): Nandakumar, Renu; Afshari, Hessam; Bruley, Duane F.

CORPORATE SOURCE: College of Engineering, University of Maryland Baltimore County, Baltimore, MD, 21250, USA
SOURCE: Advances in Experimental Medicine and Biology (2003), 540(Oxygen Transport to Tissue XXV), 191-199
CODEN: AEMBAP; ISSN: 0065-2598
PUBLISHER: Kluwer Academic/Plenum Publishers
DOCUMENT TYPE: Journal
LANGUAGE: English
AB An attempt was made to analyze the equilibrium adsorption isotherms for protein C (PC) on metal, Cu(II)-chelated IDA to explore in depth the various interactions that govern the retention and release process which will in turn provide an insight crucial for designing an effective immobilized metal affinity chromatog
. (IMAC) separation process for PC at a preparative scale and predicting its performance in advance. The non-chromatog. batch type equilibrium binding of protein interaction with immobilized ligands was investigated. This approach is simple in design, requires fewer assumptions, flexible in choosing exptl. conditions, requires less proteins and is readily amenable to the rapid evaluation of both stationary and mobile phase manipulations when compared to frontal chromatog. Batch type equilibrium binding has been widely used for determining adsorption parameters for ion exchange, affinity and immobilized metal ion affinity adsorbents. Results of this study will aid immensely in the determination of binding capacity. This result will also help in the selection, optimization, and design of the IMAC process to effectively and economically sep. PC from homologous blood factors, for the treatment of deep vein thrombosis, skin necrosis, sepsis, etc.
REFERENCE COUNT: 22 THERE ARE 22 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 24 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2003:294415 CAPLUS
DOCUMENT NUMBER: 139:272539
TITLE: Purification of recombinant BtpA and Ycf3, proteins involved in membrane protein biogenesis in Synechocystis PCC 6803
AUTHOR(S): Schwabe, Tatjana M. E.; Gloddek, Kirsten; Schluesener, Daniela; Kruip, Jochen
CORPORATE SOURCE: Plant Biochemistry, Faculty of Biology, Ruhr-University Bochum, Bochum, 44801, Germany
SOURCE: Journal of Chromatography, B: Analytical Technologies in the Biomedical and Life Sciences (2003), 786(1-2), 45-59
CODEN: JCBAAI; ISSN: 1570-0232
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The gene products Ycf3 (hypothetical chloroplast open reading frame) and BtpA (biogenesis of thylakoid protein) are thought to be involved in the biogenesis of the membrane protein complex photosystem I (PSI) from Synechocystis PCC 6803. PSI consists of 12 different subunits and binds more than 100 cofactors, making it a model protein to study different aspects of membrane protein biogenesis. For a detailed biophys. characterization of Ycf3 and BtpA pure proteins must be available in sufficient quantities. Therefore we cloned the corresponding genes into expression vectors. To facilitate purification we created His-tagged versions of Ycf3 and BtpA in addition to the unmodified forms. Immobilized metal affinity chromatog. (IMAC) yielded His-tagged proteins which were used for the production of antibodies. Purification strategies for non-tagged proteins could also be established: Ycf3 could be purified in

soluble form using a two-step purification in which ammonium sulfate precipitation was combined with anion-exchange chromatog. (IEC). BtpA had to be purified from inclusion bodies by two-consecutive IEC steps under denaturing conditions. An optimized refolding protocol was established that yielded pure BtpA. In all cases, MALDI-TOF peptide mass fingerprinting (PMF) was used to confirm protein identity. Initially, size exclusion chromatog. and CD-spectroscopy were used for biophys. characterization of the proteins. Both Ycf3 and BtpA show homo-oligomerization in vitro. In summary, purification protocols for Ycf3 and BtpA have been designed that yield pure proteins which can be used to probe the mol. function of these proteins for membrane protein biogenesis.

REFERENCE COUNT: 17 THERE ARE 17 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 26 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN

ACCESSION NUMBER: 2001:97903 CAPLUS

DOCUMENT NUMBER: 134:248994

TITLE: Characterization of metal affinity of green fluorescent protein and its purification through salt promoted, immobilized metal affinity chromatography

AUTHOR(S): Li, Y.; Agrawal, A.; Sakon, J.; Beitle, R. R.

CORPORATE SOURCE: Department of Chemical Engineering, University of Arkansas, Fayetteville, AR, 72701, USA

SOURCE: Journal of Chromatography, A (2001), 909(2), 183-190
CODEN: JCRAEY; ISSN: 0021-9673

PUBLISHER: Elsevier Science B.V.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Immobilized metal affinity chromatog. (IMAC) was investigated as a method of recovery for green fluorescent protein (GFPuv). It was found that in the absence of genetic modification to enhance metal affinity, GFPuv displayed strong metal affinity to Cu(II) and Ni(II), and weak or negligible affinity to Zn(II) and Co(II). Changes in the mobile phase NaCl concentration during Ni(II)-IMAC strongly affected purity and yield of GFPuv, with fine resolution under higher NaCl concns. Finally, IMAC via Cu(II) and Zn(II) with intervening diafiltration was used to recover GFPuv with high yield and purity.

REFERENCE COUNT: 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 27 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN

ACCESSION NUMBER: 2000:187699 CAPLUS

DOCUMENT NUMBER: 132:307303

TITLE: Immobilized metal affinity chromatography of β-galactosidase from unclarified Escherichia coli homogenates using expanded bed adsorption

AUTHOR(S): Clemmitt, R. H.; Chase, H. A.

CORPORATE SOURCE: Department of Chemical Engineering, University of Cambridge, Cambridge, UK

SOURCE: Journal of Chromatography, A (2000), 874(1), 27-43
CODEN: JCRAEY; ISSN: 0021-9673

PUBLISHER: Elsevier Science B.V.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The development of an expanded bed process for the direct extraction and partial purification of β-galactosidase from unclarified Escherichia coli homogenates using its natural affinity for metal loaded

STREAMLINE Chelating is described. Small packed beds were used to determine the effect of chelated metal ion (Cu2+, Ni2+, Co2+ or Zn2+), loading pH and ionic strength on the selective binding capacity, and recovery of β-galactosidase from clarified homogenates. An elution protocol was developed using the competitive displacer, imidazole, to recover β-galactosidase in 87% yield and 3.4-fold purification These results were then used to develop a separation for the recovery of β-galactosidase from unclarified homogenates in a 2.5-cm diameter expanded bed. Although Ni2+ loaded STREAMLINE Chelating had a 5% dynamic capacity for β-galactosidase of just 118 U ml-1 (0.39 mg ml-1), the low capacity was thought to be due to the large size of the target (464,000) relative to the exclusion limit of the macroporous adsorbent. Despite this low capacity, Ni2 STREAMLINE Chelating was used successfully to recover β-galactosidase from an unclarified homogenate in 86.4% yield and at 5.95-fold purification The degree of purification relative to a com. standard, as assessed using the purification factor and sodium dodecyl sulfate-polyacrylamide gel electrophoresis was high suggesting that this pseudo-affinity procedure compared favorably with alternative methods.

REFERENCE COUNT: 62 THERE ARE 62 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 28 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN

ACCESSION NUMBER: 2000:55130 CAPLUS

DOCUMENT NUMBER: 132:191327

TITLE: Comparison of different transition metal ions for immobilized metal affinity chromatography of selenoprotein P from human plasma

AUTHOR(S): Sidenius, U.; Farver, O.; Jons, O.; Gammelgaard, B.

CORPORATE SOURCE: Department of Analytical and Pharmaceutical Chemistry, The Royal Danish School of Pharmacy, Copenhagen, DK-2100, Den.

SOURCE: Journal of Chromatography, B: Biomedical Sciences and Applications (1999), 735(1), 85-91
CODEN: JCBBEP; ISSN: 0378-4347

PUBLISHER: Elsevier Science B.V.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Cu2+, Ni2+, Zn2+, Co2+ and Cd2+ were evaluated in metal ion affinity chromatog. for enrichment of selenoprotein P, and immobilized Co2+ affinity chromatog. was found to be the most selective chromatog. method. The chromatog. was performed by fast protein liquid chromatog. and the fractionation was followed by anal. of the collected fractions for selenium by inductively coupled plasma mass spectrometry. By the combination of immobilized Co2+ affinity chromatog. and heparin affinity chromatog. a simple method was developed yielding a 14 800-fold enrichment of selenoprotein P. The purity of the protein was determined by SDS-PAGE and by sequencing from polyvinylidene difluoride blots of SDS-PAGE gels.

REFERENCE COUNT: 29 THERE ARE 29 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 29 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN

ACCESSION NUMBER: 1999:206234 CAPLUS

DOCUMENT NUMBER: 130:310701

TITLE: Purification of papain by immobilized metal affinity chromatography (IMAC) on chelating carboxymethyl cellulose

AUTHOR(S): D'Souza, Fatima; Lali, Arvind

CORPORATE SOURCE: Chemical Engineering Division, Department of Chemical Technology, University of Mumbai, Mumbai, 400 019, India

SOURCE: Biotechnology Techniques (1999), 13(1),

59-63
CODEN: BTECE6; ISSN: 0951-208X
PUBLISHER: Kluwer Academic Publishers
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Chelating CM-cellulose was prepared in bead form by immobilizing iminodiacetic acid on CM-cellulose which was earlier crosslinked and activated by epichlorohydrin. The prepared matrix was used to purify papain by a factor of 2.6 from com. papain, and by a factor of 4 from papaya latex by batch adsorption and immobilized metal affinity chromatog. resp. Purification factors obtained were equal in batch mode and double in column mode, to purifications obtained on Chelating Sepharose Fast Flow. Flow rates up to 38 mL/cm2 h were easily possible on the prepared chelating CM-cellulose.
REFERENCE COUNT: 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 32 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1997:707622 CAPLUS
DOCUMENT NUMBER: 128:72469
TITLE: Selection of optimum affinity tags from a phage-displayed peptide library. Application to immobilized copper(II) affinity chromatography
AUTHOR(S): Patwardhan, A. V.; Goud, G. N.; Koepsel, R. R.; Ataai, M. M.
CORPORATE SOURCE: Department of Chemical Engineering and the Center for Biotechnology and Bioengineering, University of Pittsburgh, 300 Technology Drive, Pittsburgh, PA, 15219, USA
SOURCE: Journal of Chromatography, A (1997), 787(1 + 2), 91-100
CODEN: JCRAEY; ISSN: 0021-9673
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Immobilized metal affinity chromatog. (IMAC) is a versatile tool for the purification of proteins with affinity for immobilized metals. Moreover, this technique has also been used for the separation of proteins that do not exhibit significant metal affinity in the native form, by their fusion to a short metal-binding peptide (a tail), most commonly, a sequence consisting of six adjacent histidine residues (His6). A phage-displayed random hexamer library is used to select for peptides with affinity for immobilized copper. The study follows our previous investigation in which a stringent selection protocol led to the selection of only one copper-binding peptide containing two histidines. The less stringent conditions employed in this work resulted in the selection of a more diverse population of peptides, but again, dominated by peptides containing two histidines (13 out of 19). The prevalence of peptides with two histidines, in contrast to peptides with a higher number of histidines (e.g. His6 or HHHMVH), is explained based on the differences in the pH dependence of their affinity for copper. As discussed, the selected peptides with two histidines will be superior affinity tails than peptides with a higher histidine content (e.g.His6). Moreover, a peptide with a single histidine but with a very high copper affinity, is also identified. Its high copper affinity is related to the presence of several hydrophobic residues in the neighborhood of histidine. Chromatog. of human interleukin-1β (hIL-1β) and several other proteins containing a single surface-exposed histidine surrounded by several hydrophobic residues confirmed that such a sequence could also serve as a very effective metal binding domain for protein purification using immobilized copper(II) columns.

L3 ANSWER 33 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1996:615860 CAPLUS
DOCUMENT NUMBER: 126:3574
TITLE: Purification of human erythrocyte acetylcholinesterase by immobilized metal affinity chromatography
AUTHOR(S): Il'ina, A. V.; Bannikova, G. E.; Varlamov, V. P.; Agabekyan, R. S.
CORPORATE SOURCE: "Bioinzheneriya" Tsentr, Moscow, 117312, Russia
SOURCE: Prikladnaya Biokhimiya i Mikrobiologiya (1996), 32(4), 389-392
CODEN: PBMIAK; ISSN: 0555-1099
PUBLISHER: MAIK Nauka
DOCUMENT TYPE: Journal
LANGUAGE: Russian
AB Acetylcholinesterase was purified on columns of iminodiacetic acid-agarose charged with Cu2+, Zn2+, and Ni2+. The best results (14-fold purification and >30% activity yield) were achieved with Zn2+ as complexing metal. The preparation had a specific activity of approx. 7 U. The purity was tested by polyacrylamide gel electrophoresis under denaturing conditions.

L3 ANSWER 34 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1997:335335 CAPLUS
DOCUMENT NUMBER: 127:62672
TITLE: Salicylaldehyde-metal-amino acid ternary complex: a new tool for immobilized metal affinity chromatography
AUTHOR(S): Leibler, D.; Rabinkov, A.; Wilchek, M.
CORPORATE SOURCE: Dep. Biophysics, Weizmann Inst. Sci., Rehovot, 76100, Israel
SOURCE: Journal of Molecular Recognition (1996), 9(5/6), 375-382
CODEN: JMORE4; ISSN: 0952-3499
PUBLISHER: Wiley
DOCUMENT TYPE: Journal
LANGUAGE: English
AB An immobilized salicylaldehyde (sal) was used to build various salicylaldehyde-copper-amino acid (Sal-Cu-AA) complexes which are stable at a range of pH values (2.0-11.0). The complexes were found to bind protein mols. as IMAC resins. Thirteen proteins were examined for their binding to a Sal-Cu-Gly column. The efficacy of the Sal-Cu-AA resin for protein separation were demonstrated by two examples. The first was a new purification process for garlic lectins from garlic crude extract It seems that in this case the Sal-Cu-AA resins were more selective than IDA resin. The second was immobilization of Con A on the resin and using the immobilized Con A for affinity chromatog. of mannose-rich glycoprotein ovalbumin. The Con A could be later eluted with EDTA or imidazole and the Sal-containing polymer could be recharged again for further use.
REFERENCE COUNT: 37 THERE ARE 37 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 35 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1996:297323 CAPLUS
DOCUMENT NUMBER: 125:7730
TITLE: Fractionation of perforin and granzymes by immobilized metal affinity chromatography (IMAC)
AUTHOR(S): Winkler, Ulrike; Pickett, Timothy M.; Hudig, Dorothy
CORPORATE SOURCE: Cell and Molecular Biology Program, Department of Microbiology and School of Veterinary Medicine, School of Medicine and Collage of Agriculture, University of Nevada, Reno, NV, USA
SOURCE: Journal of Immunological Methods (1996),

191(1), 11-20
CODEN: JIMMBG; ISSN: 0022-1759
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Cytotoxic lymphocytes and natural killer cells kill their targets by releasing pore-forming granules or by Fas ligand-Fas initiated death. The granules contain the pore-forming protein perforin, proteoglycan and multiple serine proteases termed granzymes. In this paper we describe two options for isolating perforin and granzymes. Both options sep. the proteins by their ability to bind to immobilized metal affinity chromatog. (IMAC) columns. The first option, with Cu2+ as the metal (Cu2+-IMAC), separates both perforin and granzymes while the second, with Co2+ as the metal (Co2+-IMAC), separates only perforin. After Cu2+-IMAC perforin is >20-fold enriched with excellent recovery of lytic activity. Only two proteins are substantial contaminants. After Cu2+-IMAC, the perforin is dilute and requires concentration before addnl. steps of purification The second option, with Co2+ as the metal (Co2+-IMAC), yields perforin that is concentrated in a sharp peak. The concentrated perforin is immediately suitable for further purification The first option, with Cu2+, isolates the granzymes while the second option, Co2+-IMAC, does not. After isolation, the perforin lytic and granzyme activities are stable for weeks at 4°C, an advantage to previous isolation methods for these proteins. The excellent recoveries of perforin and granzymes also indicate that these proteins are less than 4% and 15% of the total lymphocyte granule protein, resp.

L3 ANSWER 36 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1995:797453 CAPLUS
DOCUMENT NUMBER: 123:193026
TITLE: Method for purification of a mixture of hydroxamate derivatized protein and native protein
INVENTOR(S): Wikstroem, Per
PATENT ASSIGNEE(S): Pharmacia AB, Swed.
SOURCE: PCT Int. Appl., 14 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9514035	A1	19950526	WO 1994-SE1088	19941117 <--
W: AU, BG, BR, CA, CN, CZ, FI, HU, JP, KR, LT, LV, NO, NZ, PL, RU, SK, UA, US				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
CA 2175591	A1	19950526	CA 1994-2175591	19941117 <--
AU 9510814	A	19950606	AU 1995-10814	19941117 <--
AU 683966	B2	19971127		
EP 738276	A1	19961023	EP 1995-901671	19941117 <--
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
JP 09505078	T	19970520	JP 1994-514396	19941117 <--
US 5912329	A	19990615	US 1996-635908	19960425 <--

PRIORITY APPLN. INFO.: SE 1993-3822 A 19931118
WO 1994-SE1088 W 19941117

AB The invention relates to a method for purification of a mixture of hydroxamate derivatized protein/proteins and native protein which is characterized by treating the mixture with immobilized metal and thereby adsorbing the hydroxamate derivatized protein/proteins on the immobilized metal and recovering the native protein. The protein could be

insulin-like growth factor I. It also relates to a process for the production of a native protein which is characterized by expression of the protein as a fusion protein, cleavage of the fusion protein by hydroxylamine, separation of native protein from hydroxamate derivatized protein by adsorbing the hydroxamate derivatized protein on immobilized metal and directly recovering the native protein. The use of immobilized metal affinity chromatog. for separation of native protein from hydroxamate derivatized protein is also claimed.

L3 ANSWER 37 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1994:318517 CAPLUS
DOCUMENT NUMBER: 120:318517
TITLE: Immobilized metal affinity membrane adsorbers as stationary phases for metal interaction protein separation
AUTHOR(S): Reif, Oscar Werner; Nier, Volker; Bahr, Ute; Freitag, Ruth
CORPORATE SOURCE: Inst. Tech. Chem., Univ. Hannover, Hannover, 30167, Germany
SOURCE: Journal of Chromatography, A (1994), 664(1), 13-25
CODEN: JCRAEY; ISSN: 0021-9673
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Novel immobilized metal affinity membrane adsorbers (IMA-MA) were studied for potential use as stationary phases for protein separation Protein adsorption on IMA-MA loaded with Cu(II), Ni(II), Zn(II) and Co(II) ions was compared as a function of the flow-rate and the ionic strength of the elution buffer. To exclude the possibility of mixed-mode interaction in the expts., the binding of proteins similar in terms of hydrophobicity, isoelec. point, size and mass-to-charge ratio but differing in their number of surface histidine residues was investigated. Matrix-assisted laser desorption/ionization mass spectrometry was used to distinguish between these proteins in the eluted fractions. Salt concentration of at least 0.5 M NaCl and flow-rates below 2 mL min-1 were found suitable to ensure an adsorption mechanism based on affinity interaction between the proteins and the chelated metal ions. In an application study, the IMA-AA and conventional chelating Sepharose fast flow columns were compared for the isolation of a recombinant fusion protein (EcoR V), which carried a polyhistidine sequence (HIS6-tag) at the N-terminus.

L3 ANSWER 38 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1992:403117 CAPLUS
DOCUMENT NUMBER: 117:3117
TITLE: Resolution of three forms of superoxide dismutase by immobilized metal affinity chromatography
AUTHOR(S): Michalski, Wojtek P.
CORPORATE SOURCE: Anim. Health Res. Lab., CSIRO, Parkville, 3052, Australia
SOURCE: Journal of Chromatography (1992), 576(2), 340-5
CODEN: JOCRAM; ISSN: 0021-9673
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A new method for the separation of 3 forms of superoxide dismutase (SOD) using immobilized metal affinity chromatog. (IMAC) is reported. Fe-, Mn- and Cu/Zn-containing SODs were eluted sequentially from a Cu2+-IMAC column with an increasing gradient of a counterion (NH4+) run in combination with an increasing pH gradient (6.8-7.8). The combined gradient elution method resulted in separation of SODs with high resolution, the 3 proteins being eluted in electrophoretically homogeneous forms. Similar preparation could not be

achieved by either increasing gradient of a counterion or decreasing pH gradients used sep. The described methodol. was successfully applied for the separation of 3 SODs from a protozoan parasite, Eimeria tenella, indicating that this combined gradient elution system for IMAC offers new possibilities for the high-resolution separation of proteins exhibiting only minor differences in their amino acid composition and structure.

L3 ANSWER 39 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1990:94793 CAPLUS
DOCUMENT NUMBER: 112:94793
TITLE: Rapid analytical and preparative scale immobilized metal affinity chromatography to purify α-lactalbumin from milk whey
AUTHOR(S): Reid, Terrence S.; Stancavage, Ann M.
CORPORATE SOURCE: Supelco, Bellefonte, PA, 16823-0048, USA
SOURCE: BioChromatography (1989), 4(5), 262-5
CODEN: BCHREF; ISSN: 0888-4404
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Anal. and preparative scale purifns. of α-lactalbumin from bovine milk whey were performed by immobilized metal affinity chromatog. using immobilized iminodiacetic acid bound with Cu(II) as the stationary phase. An anal. scale separation was developed using a small HPLC column packed with 10-μ-diameter vinyl polymer particles having 1000-Å-diameter pores. Scale up to preparative sepns. was readily accomplished using totally porous, spherical, 40- to 90-μ-diameter vinyl polymer particles packed in a 2.5-cm ID glass column.

L3 ANSWER 40 OF 45 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1988:34287 CAPLUS
DOCUMENT NUMBER: 108:34287
TITLE: Separation of ovotransferrin from egg white by immobilized metal affinity chromatography
AUTHOR(S): Al-Mashikhi, S. A.; Nakai, Shuryo
CORPORATE SOURCE: Dep. Food Sci., Univ. British Columbia, Vancouver, BC, V6T 2A2, Can.
SOURCE: Agricultural and Biological Chemistry (1987), 51(11), 2881-7
CODEN: ABCHA6; ISSN: 0002-1369
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Immobilized metal affinity chromatog. (IMAC) was used to sep. ovotransferrin (OVT) by a single chromatog. step. Ovotransferrin was bound strongly to immobilized copper ions, while other components of egg white passed through the column. Using acidic pH and/or a strong competing solute, imidazole, ovotransferrin was recovered in 94-98% pure form as indicated by SDS-polyacrylamide gel electrophoresis and immunoelectrophoresis. The elution profiles from the IMAC column indicated the presence of two forms of ovotransferrin. The capacity of the IMAC column was 20 mg OVT/mL copper loaded gel. Saturation of the metal binding sites of ovotransferrin with Fe2+ and Cu2+ did not interfere with the binding of ovotransferrin to the IMAC column. However, modification of the histidine residues in ovotransferrin with di-Et pyrocarbonate almost completely destroyed the binding to the IMAC column.

L3 ANSWER 42 OF 45 EMBASE COPYRIGHT (c) 2007 Elsevier B.V. All rights reserved on STN
ACCESSION NUMBER: 2001096185 EMBASE
TITLE: Efficient two-step chromatographic purification of penicillin acylase from clarified Escherichia coli ultrasonic homogenate.

AUTHOR: Sanchez J.; Verdoni N.; Fitton V.; Santarelli X.
CORPORATE SOURCE: X. Santarelli, E. Superieure Technol./Biomolecules, CNRS-UMR 5544, Univ. V. Segalen Bordeaux 2, 146 Rue Leo Saignat, 33076 Bordeaux Cedex, France. xavier.santarelli@estbb.u-bordeaux2.fr
SOURCE: Journal of Chromatography B: Biomedical Sciences and Applications, (25 Mar 2001) Vol. 753, No. 1, pp. 45-50.
Refs: 26
ISSN: 1387-2273 CODEN: JCBBEP
PUBLISHER IDENT.: S 0378-4347(00)00529-6
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Conference Article; (Conference paper)
FILE SEGMENT: 004 Microbiology: Bacteriology, Mycology, Parasitology and Virology
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 12 Apr 2001
Last Updated on STN: 12 Apr 2001

AB A two-step chromatographic purification procedure from clarified Escherichia coli ultrasonic homogenate was evaluated. The capture step included immobilized metal affinity chromatography with Cu(2+) as metal ion. Two elution methods were performed: 1 M NH(4)Cl and 0.01 M imidazole. Respectively, we obtained a different purification fold (16.5 to 3.15) and a similar result for the recovery of activity (90-99%). The best elution method was chosen for the procedure. The second step, hydrophobic interaction chromatography, gave a 3.8-fold purification with 77.7% of activity. The total procedure gave a 66-fold purification in relation to the initial crude extract with 70% for the recovery of activity and was performed without any conditioning step and at the same pH value. .COPYRGT. 2001 Elsevier Science B.V.

L3 ANSWER 43 OF 45 EMBASE
ACCESSION NUMBER: 1998156861 EMBASE
TITLE: Purification and characterization of lymphocyte chymase I, a granzyme implicated in perforin-mediated lysis.
AUTHOR: Woodard S.L.; Fraser S.A.; Winkler U.; Jackson D.S.; Kam C.-M.; Powers J.C.; Hudig D.
CORPORATE SOURCE: D. Hudig, Mail Code 320, School of Medicine, University of Nevada, Reno, NV 89557, United States. hudig@med.unr.edu
SOURCE: Journal of Immunology, (15 May 1998) Vol. 160, No. 10, pp. 4988-4993.
Refs: 40
ISSN: 0022-1767 CODEN: JOIMA3
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 026 Immunology, Serology and Transplantation
029 Clinical and Experimental Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 11 Jun 1998
Last Updated on STN: 11 Jun 1998

AB One mechanism of killing by cytotoxic lymphocytes involves the exocytosis of specialized granules. The released granules contain perforin, which assembles into pores in the membranes of cells targeted for death. Serine proteases termed granzymen are present in the cytotoxic granules and include several chymases (with chymotrypsin-like specificity of cleavage). One chymase is selectively reactive with an inhibitor, Biotinyl-Aca-Aca-Phe- Leu-Phe(P)(OPh)(2), that blocks perforin lysis. We

report the purification and characterization of this chymase, lymphocyte chymase I, from rat natural killer cell (RNK)-16 granules. Lymphocyte chymase I is 30 kDa with a pH 7.5 to 9 optimum and primary substrate preference for tryptophan, a preference distinct from rat mast cell chymases. This chymase also reacts with other selective serine protease inhibitors that block perforin pore formation. It elutes by Cu(2+)-immobilized metal affinity chromatography with other granzymes and has the N-terminal protein sequence conserved among granzymes. Chymase I reduces pore formation when preincubated with perforin at 37°C. In contrast, addition of the chymase without preincubation had little effect on lysis. It should be noted that the perforin preparation contained sufficient residual chymase activity to support lysis. Thus, the reduction of lysis may represent an effect of excess prolytic chymase I or a means to limit perforin lysis of bystander cells. In contrast, other chymases and granzyme K were without effect when added to perforin during similar preincubation. Identification of the natural substrate of chymase I will help resolve how it regulates perforin-mediated pore formation.

L3 ANSWER 44 OF 45 EMBASE

ACCESSION NUMBER: 1999063876 EMBASE
TITLE: Chromatographic behaviour of amidases from strains of Pseudomonas aeruginosa on immobilized metal chelates.
AUTHOR: Martinho F.; Massano R.; Karmali A.; Brown P.R.
CORPORATE SOURCE: A. Karmali, Laboratorio de Engenharia Bioquimica, Departamento de Engenharia Quimica, Instituto Superior Engenharia Lisboa, Rua Conselheiro Emidio Navarro, 1900 Lisboa, Portugal
SOURCE: International Journal of Bio-Chromatography, (1998) Vol. 4, No. 2, pp. 101-114.
Refs: 32
ISSN: 1068-0659 CODEN: IJOBEQ
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical and Experimental Biochemistry
004 Microbiology: Bacteriology, Mycology, Parasitology and Virology
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 25 Feb 1999
Last Updated on STN: 25 Feb 1999
AB The amidase from a wild-type strain 8602 of Pseudomonas aeruginosa bound only to Cu(II)-IDA agarose column whereas the enzyme from the mutant strain A13 bound to Cu(II)-IDA as well as to Ni(II)-IDA agarose columns. Both enzymes bound to Cu(II)-IDA agarose columns and were eluted by imidazole in a catalytically inactive form which was detected by ELISA using monoclonal antibodies. The inactive amidases were reactivated by the addition of dithiothreitol. Both enzymes did not bind to Zn(II)-IDA columns since most of the activity was found in fractions at the washing step. Amidase from a mutant strain A13 of Pseudomonas aeruginosa was purified in one step by immobilized metal affinity chromatography (IMAC) on metal ion-IDA agarose charged either with Cu(II) or Ni(II) ions with a recovery of enzyme activity in the range of 72-93%. The purified enzyme preparation had a specific activity in the range of 6.6-7.4 U/mg protein and was apparently homogeneous on SDS-PAGE and native PAGE. The effect of several metal ions on amidase activity revealed that Cu(II) ions act as powerfull inhibitors, whereas Ni(II), Zn(II) and Co(II) ions did not alter the enzyme activity.

L3 ANSWER 45 OF 45 EMBASE

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ACCESSION NUMBER: 1997245275 EMBASE
TITLE: Perforin-enhancing protein, a low molecular weight protein of cytotoxic lymphocyte granules, enhances perforin lysis.
AUTHOR: Winkler U.; Fraser S.A.; Hudig D.
CORPORATE SOURCE: D. Hudig, Cell and Molecular Biology Program, Department of Microbiology, University of NV, NV, United States. hudig@med.unr.edu.
SOURCE: Biochemical and Biophysical Research Communications, (9 Jul 1997) Vol. 236, No. 1, pp. 34-39.
Refs: 45
ISSN: 0006-291X CODEN: BBRCA9
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 026 Immunology, Serology and Transplantation
029 Clinical and Experimental Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 29 Aug 1997
Last Updated on STN: 29 Aug 1997

AB Perforin is a 68 kD protein found in the granules of cytotoxic lymphocytes and is used by lymphocytes to form lethal pores in the membranes of the cells they kill. We and others have found that when perforin is purified, its lytic activity is markedly reduced. ELISAs indicated that our final recovery of perforin protein was excellent, We decided to determine if depletion of other granule proteins contributed to the loss of lytic activity. We isolated perforin to the point where lytic activity was diminished and added back granule proteins that had no lytic activity or detectable (antigenic) perforin. Perforin was isolated by Cu (2+)-immobilized metal affinity chromatography (IMAC) followed by phenyl-Superose hydrophobic interaction chromatography (HIC). Its lytic activity was enhanced by a low molecular weight (< 15 kD) protein, perforin enhancing protein (PEPr). We have isolated PEPr by two methods, HIC and MonoQ. Nonlytic PEPr restored perforin to close to its original lytic activity. A protein similar if not identical to PEPr was also detectable as an (125)I-labeled protein associated with lytic perforin. We propose that PEPr acts in conjunction with]perforin to form lethal pores and suggest that PEPr may be the rat equivalent of the human cytotoxic lymphocyte protein, granulysin.

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